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Fungal and bacterial growth in floor dust at elevated relative humidity levels

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Abstract

Under sustained, elevated building moisture conditions, bacterial and fungal growth occurs. The goal of this study was to characterize microbial growth in floor dust at variable equilibrium relative humidity (ERH) levels. Floor dust from one home was embedded in coupons cut from a worn medium-pile nylon carpet and incubated at 50%, 80%, 85%, 90%, 95%, and 100% ERH levels. Quantitative PCR and DNA sequencing of ribosomal DNA for bacteria and fungi were used to quantify growth and community shifts. Over a 1-wk period, fungal growth occurred above 80% ERH. Growth rates at 85% and 100% ERH were 1.1×10^4 and 1.5×10^5 spore equivalents d^{-1} mg $dust^{-1}$, respectively. Bacterial growth occurred only at 100% ERH after 1 wk (9.0×10^4 genomes d^{-1} mg $dust^{-1}$). Growth resulted in significant changes in fungal ($P<.00001$) and bacterial community structure ($P<.00001$) at varying ERH levels. Comparisons between fungal taxa incubated at different ERH levels revealed more than 100 fungal and bacterial species that were attributable to elevated ERH. Resuspension modeling indicated that more than 50% of airborne microbes could originate from the resuspension of fungi grown at ERH levels of 85% and above.

KEYWORDS

bacteria, DNA sequencing, exposure, fungi, indoor microbiome, moisture, resuspension

1 | INTRODUCTION

The origins of fungi and bacteria in the floor and surface dusts of buildings include deposition of species that originated from outdoor air, microbiota shed from humans, pets, and pests, growth on materials, and tracked in soil.[1–4] It remains unclear whether floor dust acts simply as a fungal and bacterial depository of these sources, or if an active, functioning microbial ecology exists. In buildings, water availability is considered the major limitation to microbial growth.[5] Research on fungal growth in building materials is extensive, and prior results indicate that at common indoor temperatures ranging from 20 to 25°C, lower limits of equilibrium relative humidity (ERH) that support fungal growth on wood, gypsum, and ceramics are 78%, 86%, and >90%, respectively.[6] Based on culture analyses, candidate fungi have been ascribed to moisture conditions and materials.[6,7] By contrast, the knowledge base of fungal and bacterial growth in house dust is limited, with one earlier study demonstrating production of carbon dioxide and MVOC, and growth of *Aspergillus* spp., *Eurotium* spp., and *Penicillium* spp. above RH conditions of 84%.[8]

Microbial growth on dust holds added significance due to the potential for dust-borne microbes to become resuspended into indoor air and produce an inhalation exposure. Resuspension attributable to human occupancy is a well-known mechanism for aerosolizing microbes.[9,10] Prior studies in homes and schools indicate that indoor air to outdoor air ratios for both fungi and especially bacteria are elevated above one when rooms are occupied, and that resuspension from flooring is the primary mechanism,[3,11,12] Resuspension has also been associated with increased exposure to fungal and other aeroallergens.[13,14]

The goal of this study was to characterize microbial growth in floor dust at variable ERH levels. Floor dust was embedded on coupons of worn, medium-pile carpet collected from the same home, and these coupons were then incubated at ERH levels ranging from 50% to 100%. To describe how bacterial and fungal communities change with ERH, microbes were tracked through quantitative PCR and DNA sequencing of 16SrDNA genes from bacteria and internal transcribed spacer (ITS) DNA from fungi. This work extends the study of microbial growth on building materials by the inclusion of floor dust, consideration of bacteria and fungi, and utilization of DNA-based approaches to characterize microbial communities.

Practical Implications

This study described and quantified fungal and bacterial community changes in floor dust under elevated and continuous ERH. The utilization of DNA sequencing to track bacterial and fungal growth on floor dust provides new information on the diversity of microbes that may result due to moisture in buildings. Through resuspension modeling, this work describes how microbial growth in floor dust can increase airborne exposure to moisture-associated bacteria and fungi. Combining the large increase in microbes at ERH levels >80% and the strong source term of occupancy-driven resuspension suggests that growth on dust embedded in carpets may be a significant factor shaping human exposure in buildings under continuous, elevated RH.

2 | METHODS

2.1 | Carpet

Medium-pile, nylon carpet was collected in September 2014. This carpet was installed in approximately 2004 in a single-family home in eastern Massachusetts, USA. There was no known history of water damage or mold growth in the home, and occupants included one adult and one indoor cat. Coupons measuring 10 cm × 10 cm (approximately 30 g) were cut from the carpet, wrapped in aluminum foil, and sealed in polyethylene bags for later use. Sections were selected from a 4 m^2 high-traffic area of the carpet. Carpet coupons were stored at room temperature until use. Additional dust was collected from the occupant's vacuum cleaner bag. This dust was passed through a 300-μm sieve, mixed, and stored at room temperature. Dust and carpet were never frozen to avoid disruption of microbial communities. Nutrient analysis of the dust is described below.

2.2 | RH chamber experiments

Carpet samples impregnated with sieved dust were exposed to different levels of relative humidity to examine growth and microbial community development. Independent experiments were conducted in triplicate at 50%, 80%, 85%, 90%, 95%, and 100% relative humidity for 1 wk. At 85% and 100% relative humidity levels, experiments were extended to 6 wks. The experiments at 100% relative humidity were terminated at 3 wks due to visible fungal growth on the carpet samples. The experiment at 100% relative humidity for 1 wk was repeated (for a total of six replicates) due to high variability in the measured bacterial and fungal concentrations.

In each experiment, carpets sections were inoculated with 0.5 g of mixed, sieved dust from the home vacuum cleaner bag. Dust was embedded following ASTM method F608-13 modified by using a smaller 12-cm-long, 860-g steel pipe, while avoiding the outer 1 cm edge of the coupon. Samples were placed into autoclaved glass chambers in a 25°C incubator that consisted of 3.8-L glass jars covered with parafilm. In each chamber, the carpet coupon was placed next to a glass beaker containing 100 mL of salt solution. The water activity of the salt solution was adjusted using sodium chloride above a water activity of 0.76 and a combination of sodium chloride and magnesium chloride below 0.76.[15–17] Water activity of the salt solution and carpet dust was verified using an Aqualab Series 3 meter (Decagon Devices, Pullman, WA, USA). Real-time temperature and relative humidity were monitored in one-third of the chambers using HOBO data loggers (Onset Computer Corporation, Bourne, MA, USA). Water absorption was confirmed in all chambers based on final weight gain of the carpet coupon.

After the growth cycle, dust was collected from carpet samples using a 19 mm × 90 mm cellulose Whatman thimble inserted into a Eureka Mighty Mite with an adapter. Dust was stored at −20°C prior to DNA sequence analysis.

2.3 | Nutrient measurements

To measure soluble nutrients in the floor dust, 30 mg of dust was mixed with 20 mL deionized water and shaken for 30 minutes then filtered through a 0.45-μm syringe filter.[18] Total organic carbon was measured via TOC-V CSH analyzer (Shimadzu, Kyoto, Japan) and nitrate, phosphate, sulfate, and other dissolvable ions were measured by ion chromatography (Dionex, Sunnyvale, CA, USA). Total elemental analysis of the dust was performed at the Cornell Nutrient Analysis Laboratory using EPA Methods 3051 and 6010B; samples were analyzed via inductively coupled plasma atomic emission spectroscopy (Spectro Analytical Instrumentation, Inc., Kleve, Germany). Estimated nutrient requirements for growth were calculated using the sum of fungal and bacterial growth after 1 wk at 100% relative humidity and assuming a mass of 8.2 pg per spore for fungi and 655 fg per genome for bacteria,[11] and the elemental composition of *Saccharomyces cerevisiae*.[19]

2.4 | DNA analyses and quantitative PCR

Dust was measured into 50 mg aliquots and extracted using a modified protocol for the MoBio PowerSoil DNA extraction kit (MoBio, Carlsbad, CA, USA)[20] in a sterile, laminar flow hood. To prevent PCR inhibition, DNA extract was diluted 10× in Tris–EDTA (TE). These samples were then tested for inhibition by spiking all samples with a known concentration of *Aspergillus fumigatus* or *Bacillus atropheaus*

DNA and comparing the measured value to the expected value. Some samples from the 100% relative humidity condition demonstrated inhibition, requiring further dilution to 100× before testing confirmed a lack of inhibition.

DNA sequence library preparation and sequencing was conducted at Research and Testing Laboratory (Lubbock, TX, USA) on Illumina MiSeq (San Diego, CA, USA) equipment to generate paired-end, 2 × 250 bp reads. For fungal analysis, the internal transcribed space 1 (ITS1) DNA region was targeted.[21] This region was amplified using primers ITS1F (CTTGGTCATTTAGAGGAAGTAA) and ITS2aR (GCTGCGTTCTTCATCGATGC).[22,23] For bacterial analysis, the 16S region was amplified with primers 357wf (CCTACGGGNGGCWGCAG) and 785R (GACTACHVGGGTATCTAATCC).[24] Sequences have been archived in the European Nucleotide Archive under accession number PRJEB11542.

Quantitative PCR was used to determine total fungal, bacterial, and human cell concentration in dust as described previously.[25] Total fungal concentration was measured using the FF1/FR2 primers to the 18S rRNA gene,[26] total bacterial concentration was measured with universal primers targeting the 16S rRNA gene,[27] and total human cell concentration was measured using the intra-Yb8 primers targeting the *Alu* elements.[25,28] For absolute quantification, standard curves were created using *A. fumigatus*, *B. atropheaus*, and HEK 293 (human embryonic kidney) cells, with final values reported in spore equivalents (SE) mg^{-1} dust, genomes mg^{-1} dust, and cells mg^{-1} dust, respectively.

DNA sequence data analysis was conducted in QIIME 1.9.1.[29] Sequences were excluded if the Phred score was <20, if more than three low-quality consecutive bases occurred, or if any ambiguous base was present. Quality-trimmed sequences were clustered using UCLUST[30] at 97% similarity. Annotation was conducted through use of the GreenGenes database[31] for bacteria. For fungi, annotations were produced via BLAST version 2.2.28+[32] with the general release of the UNITE database from March 2, 2015[33] and FHiTINGS version 1.3.[34] For α diversity and rarefaction analysis, the operational taxonomic unit (OTU) tables were trimmed to the lowest number of sequences in any sample (6653 in bacteria and 18 804 in fungi). For β diversity and principal coordinate analysis (PCoA), all quality-trimmed reads were used for Morisita Horn[35] (non-phylogenetic) distance calculation for fungi and weighted/unweighted UniFrac[36] distance calculation for bacteria. Differences between groups were assessed using the ANOSIM method in QIIME with 100 000 permutations.

2.5 | Modeling resuspension

To determine whether increased dust microbial concentrations due to growth could impact airborne exposure to moisture-associated bacteria and fungi, resuspension was modeled using a steady-state material balance approach that equates outdoor air and indoor emission (resuspension) sources to ventilation and deposition losses and solves for the indoor air concentrations (Equation 1).

$$QC_{out} + rAL = QC_{in} + kC_{in}V. \quad (1)$$

Here, C_{out} is the time-averaged outdoor air concentration of bacterial (genomes·m^{-3}), or fungal spore equivalents (SE·m^{-3}); C_{in} is the time-averaged corresponding indoor concentration during the occupied period; Q is the volumetric ventilation rate ($m^3 \cdot h^{-1}$); rAL is the resuspension rate (genomes·h^{-1} or SE·h^{-1}), where r is the flooring-specific particle resuspension rate coefficient (h^{-1}), A is the actively resuspended flooring area (m^2), and L is the average floor loading (genomes·m^{-2} or SE·m^{-2}); and $kC_{in}V$ is the deposition rate (genomes·h^{-1} or SE·h^{-1}) where k is the size-specific deposition rate coefficient for bacterial cells, or fungal SE (h^{-1}) and V is the measured room volume (m^3). Outdoor fungal and bacterial concentrations were based on data from six different cities in the United States, the EU, and China[11] where the average concentrations and median geometric aerodynamic diameters, respectively, were 1.0×10^5 genomes m^{-3} and 7.4 μm for bacteria, and 1.5×10^5 SE m^{-3} and 6.6 μm for fungi. In the same United States, EU, and China study, indoor air geometric mean aerodynamic diameters of 5.5 μm for bacteria and 5.9 μm for fungi were reported.[11] Thus, a deposition rate coefficient for particles in the 4.9–9.0 μm size range of 8.6 h^{-1} and per person resuspension rate of 0.0045 h^{-1} for the 5–10 μm size range in loop-level worn carpet were used in the model.[37,38] To solve for the indoor air concentration C_{in}, the parameters Q and V were grouped into air exchange rates, which were varied from 0.1 to 6 h^{-1}, room occupancy was varied from 1 to 4 persons, and a baseline floor loading of 5×10^6 bacterial genomes m^{-2} or SE m^{-2} was used.

2.6 | Statistics

Statistical analyses were conducted in SAS, version 9.2 (SAS Institute Inc., Cary, NC, USA). Statistical methods were applied to evaluate differences in microbial growth, richness, evenness, DOC concentration, and microbial taxa based on variable relative humidity exposures.

For species/OTU comparisons, relative abundances from sequencing were multiplied by total concentration of bacterial genomes or fungal SE to yield concentrations.[39] All values for heat maps and tables were transformed using the inverse hyperbolic sine transformation to create a normalized distribution and linear data. This transformation is beneficial for use with sequencing data because it has a similar effect to logarithmic transformation but allows for values <1 and 0.[40] Reported mean values were based on "geometric mean" but used the inverse hyperbolic sine transformation in place of logarithmic to allow for these zero values. Simple linear regression with the GLM procedure was used to evaluate changes in fungal concentration, bacterial concentration, human cell concentration, fungal richness, bacterial richness, fungal evenness, and bacterial evenness with relative humidity. DOC concentrations in original dust compared to dust incubated at 100% ERH were compared with the TTEST procedure as a two-sample *t*-test. Associations between fungal and bacterial taxa were examined using the MULTTEST procedure with pfdr option on the mean among all relative humidity levels. This analysis calculates a *q*-value, which is a *P*-value that is adjusted for multiple comparisons using the false discovery rate.[41]

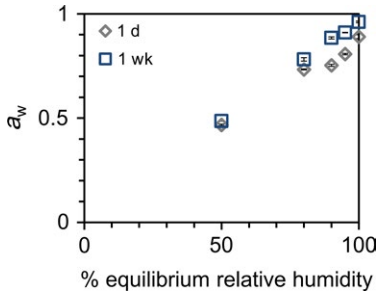

FIGURE 1 Water activity equilibration of house dust with relative humidity after 1 d and 1 wk. Error bars represent one standard error for three replicates

3 | RESULTS

3.1 | Microbial growth

Carpet coupons and dust samples readily absorbed water from the air. The water activity of dust was nearly (within 5%) equilibrated with the relative humidity in the chamber (i.e., a_w = RH) within 1 d and fully equilibrated after 1 wk (Fig. 1).

Based on the multifold increase in spore or cell concentration between t=0 and t=1 wk, both fungal and bacterial communities exhibited growth under elevated relative humidity conditions (Fig. 2). For fungi, growth increased with increasing levels of ERH for the 50%–100% range considered (simple linear regression, R^2=.62, P<.0001). At 100% relative humidity and after 1 wk of growth, there was a 27.5-fold increase in the number of fungal spores per mg dust compared to the starting concentration. Additionally, growth continued after 1 wk at 85% and 100% relative humidity (simple linear regression, R^2=.24, P=.037 and R^2=.29, P=.07, respectively). Fungal growth rates were more than 13 times higher at 100% relative humidity compared to 85% relative humidity (Table S1). For bacteria, growth was observed after 1 wk only at 100% ERH (2.7 times increase vs original levels, Fig. 2). At 100% relative humidity, this growth continued through wk three (simple linear regression, R^2=.46, P=.02) (Table S1).

Measurement of potential nutrients suggests that the major limitation to growth was available water in this home. For organic carbon, nitrate, phosphate, and sulfate, the dissolvable amount in the dust was estimated to be at least four times greater than the stoichiometric requirements to support microbial growth at 100% ERH for 1 wk (Table S2). Based on an elemental analysis of the dust, additional micronutrients were also available for growth (Table S3). Post-incubation at 100% ERH, the total amount of dissolvable organic carbon in the dust decreased (t-test, P=.0004). We also measured the concentration of human cells using qPCR as a potential nutrient source in the dust. This concentration decreased as ERH increased (R^2=.34, P=.006, Fig. S1).

3.2 | Microbial communities

After quality trimming, a total of 4 468 564 reads were included in the fungal analysis and 915 769 reads were included in the bacterial analysis. Incubation at different ERH levels was associated with changes in richness and evenness of the microbial communities (Figs 3 and S2, Table S4). For fungi, richness based on Chao1 indices and total number of OTUs decreased with increasing ERH levels (R^2=.48, P=.0005 for both). This richness trend persisted even when the communities were sampled to over 80 000 sequences per sample with singletons removed to control for sequencing errors (Fig. S2b). Fungal community evenness also decreased at increasing ERH levels as indicated by the Shannon index (R^2=.75, P<.0001). For bacteria, increases in ERH were associated with increased richness based on number of OTUs (R^2=.50, P=.0003) and Chao1 index (R^2=.34, P=.005). The Shannon index increased with increasing relative humidity (R^2=.53, P=.0002).

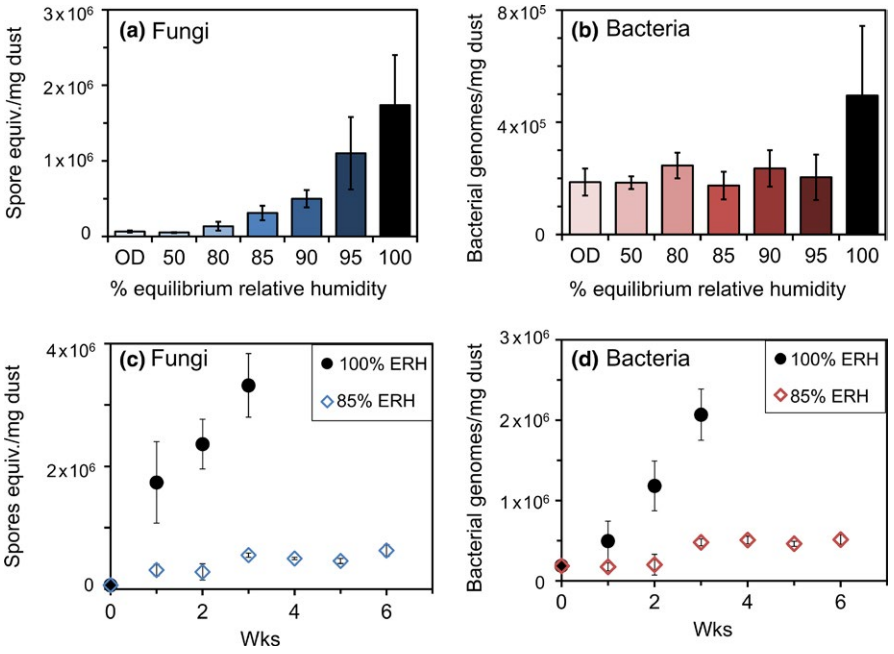

FIGURE 2 Fungal and bacterial concentrations in house dust after incubation at 50%, 80%, 85%, 90%, 95%, and 100% relative humidity for (a, b) 1 wk or (c, d) 6 wks. "OD" refers to original dust prior to incubation. Error bars represent one standard error of three replicates of different carpet coupons from the single house in this study. Six replicates were used for 100% ERH at 1 wk as noted in the Methods. Growth rates and statistics are reported in Table S1. ERH, equilibrium relative humidity

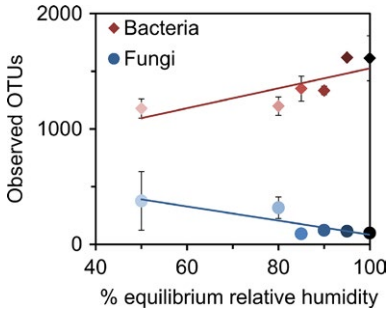

FIGURE 3 Fungal and bacterial richness (number of observed OTUs) at different equilibrium relative humidity levels after 1 wk. Error bars represent one standard error of three replicates of different carpet coupons from the single house in this study. Six replicates were used for 100% ERH at 1 wk as noted in the Methods. ERH, equilibrium relative humidity; OTU, operational taxonomic unit

In fungal communities, relative humidity was a significant driver of community composition. In PCoA, the first principal coordinate accounted for 84.0% of the variation in the data, and the second principal coordinate demonstrated an arch effect,[42] indicating a single dominant gradient in the first component (Fig. 4). ERH was strongly associated with fungal community differences among samples incubated for 1 wk (ANOSIM, R^2=.65, P<.00001, Table S5). Additionally, incubation time also resulted in statistically significant differences in microbial communities at both 85% and 100% ERH (Table S5). Fungal communities stabilized at 2 wks and later at 85% ERH and continued to change out to 3 wks at 100% ERH (Fig. S3). While increases in fungal concentration did not occur during the 1-wk incubation time at 50% ERH, we observed community differences after 1 wk of exposure to 50% ERH compared to the original dust (Fig. 4).

Fungal community comparisons were also conducted among dust incubated at the different ERH levels to determine which taxa were associated with growth at specific ERH levels. In total, 132 different fungal species and 134 different fungal genera were observed to be statistically significantly associated with ERH level (Figs 5a, S4, and S5). Tables S6, S7, S8, and S9 list fungal species, genera, class, and phyla, respectively, with statistically significant differences among ERH levels. Fungal taxa associated with relative humidity at 100% were numerically dominated by the Ascomycete *Aspergillus subversicolor* and the Basidiomycetes *Wallemia muriae* and *Wallemia sebi* (Table S6, Fig. 6). Above 80% ERH, growth was dominated by *Aspergillus*, *Penicillium*, and *Wallemia*, while below 80%, the difference in microbial community composition was due to a more diverse variety of microbes.

In bacterial communities, ERH was also associated with community composition. Communities were different from the original dust at 95% and 100% relative humidity, but not at lower levels after 1 wk (Fig. 4). There were 36 bacterial OTUs with statistically significant differences in concentration at different relative humidity levels (Figs 5b and 7, Table S10). Phyla with the highest concentration at 100% relative humidity included Firmicutes, Actinobacteria, and Gammaproteobacteria. Bacterial communities continued to change out to 3 wks at 100% ERH (Fig. S3).

3.3 | Resuspension model

Steady-state mass balance modeling using particle resuspension fractions for worn loop-level carpeting reveals the potential for large changes in indoor airborne bacterial and fungal concentrations based on the growth of these microbes in carpet dust. Under conditions including an AER of 1 h^{-1} and two occupants, our material balance model predicts that at growth due to 100% relative humidity for 1 wk, the resuspension of microbes grown in carpet dust may account for 75% of the fungal and 21% of the bacterial load in the air (Fig. 8). The contribution of floor dust growth to air increases with decreasing AER and increasing occupancy (Fig. S6). For a low range AER of 0.1 h^{-1}, and high occupancy of four persons, the contribution of indoor air communities from moisture-enabled growth becomes 95% for fungi and 57% for bacteria. For high AER (6 h^{-1}) and low occupancy (one person), these values are 22% for fungi and 2.5% for bacteria.

4 | DISCUSSION

This study comprehensively investigated the effects of elevated relative humidity on microbial growth in carpet from one home. Our results demonstrate that ERH is an important variable for defining the rates and community dynamics of microbial growth in floor dust. These effects are most profound above 80% ERH for fungi and at 100% ERH in bacteria. This study's DNA sequence-based approach allowed for tracking community features such as richness, and the culture-independent identification of taxa that were associated with

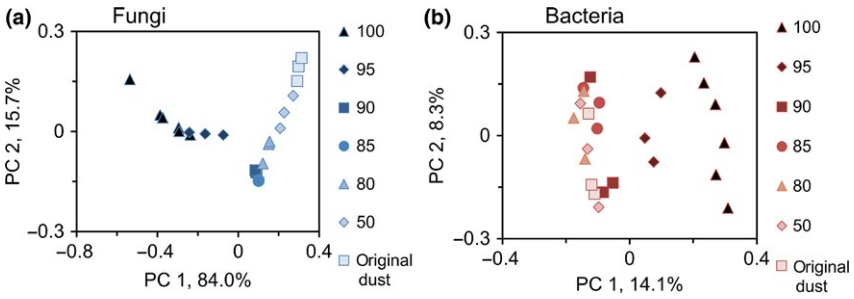

FIGURE 4 Principal coordinate plots (a) for fungi using Morisita Horn distance and (b) for bacteria using unweighted UniFrac distance. ANOSIM values are reported in Table S5 for these comparisons

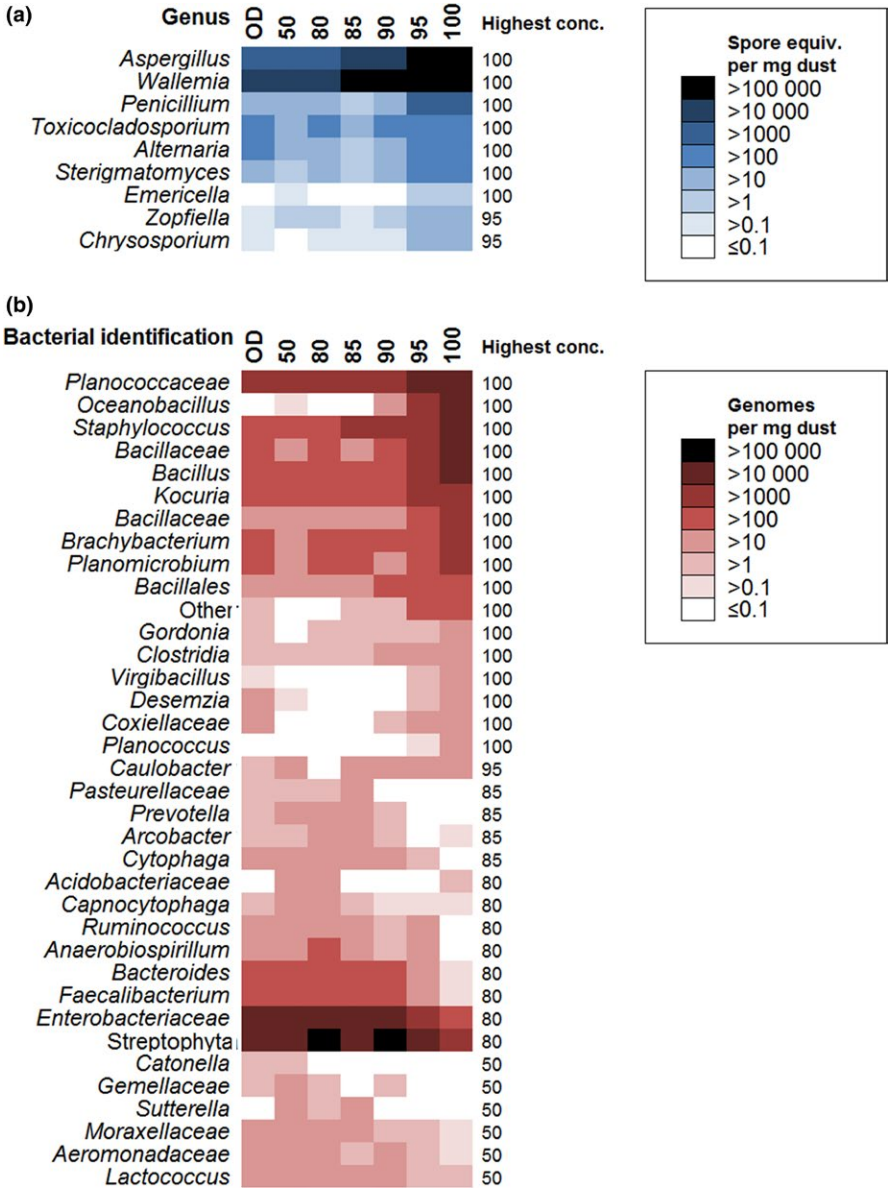

FIGURE 5 Heat map indicating the concentration of (a) fungal genera and (b) bacterial OTUs that were statistically significantly different among ERH levels. Only fungi that were most abundant at ≥90% ERH are shown here while the full fungal heat map is shown in Figs S4 and S5. Values reported on the right side of the map indicate the % ERH at which the concentration of each taxa was the highest. Statistical analysis and lists of taxa most associated with a specific ERH level are listed in Tables S6–S10. ERH, equilibrium relative humidity; OTU, operational taxonomic unit

elevated ERH levels. Resuspension modeling indicates the potential for elevated airborne exposure to moisture-associated bacteria and fungi when growth occurs in floor dust.

4.1 | Microbial communities

Culture-based analysis has provided evidence in the past that fungal species, typically in the genera *Aspergillus* and *Penicillium*, are associated with excess moisture in buildings.[43–45] However, culture-based analysis provides a more limited view of fungal diversity, especially Basidiomycota.[46] Here, DNA sequence-based approaches revealed 132 fungal species and 36 bacterial OTUs that were associated with growth on dust at different ERH conditions. At 95% and 100% relative humidity, this included commonly identified species in the genera *Aspergillus* and *Penicillium*. Members of the genus *Wallemia* were also highly enriched in the 100% ERH dust. *Wallemia* is ubiquitous in buildings due to its ability to grow at <80% ERH,[47] but reports of its growth on building materials at >95% ERH are rare, potentially due to poor growth on general fungal media. Underreporting of *Wallemia* may also be due to a decrease in relative abundance (which is typically reported, Fig. 6d) despite the increase in concentration (Fig. 6b) at increasing relative humidity. One recent study also applied DNA-based methods to characterize fungi growing on water-damaged building material and found a large diversity of microbial communities, with *Cladosporium*, *Alternaria*, *Rhodotorula*, and *Cryptococcus* and *Crivellia* as the most dominant genera on damp building materials. They noted that the community profile was heavily dependent on the specific location and material, and emphasized the need to sample multiple indoor areas when assessing mold growth.[48] Also, differences in microbial communities were observed at lower (50%) ERH levels where no increase in growth was seen (Fig. 4). Subtle changes to community composition may occur even if no statistically significant increase in total fungal or bacterial concentration is observed. Cell division has previously been observed down to 0.600–0.650 a_w in extremophiles[49,50]; thus, these

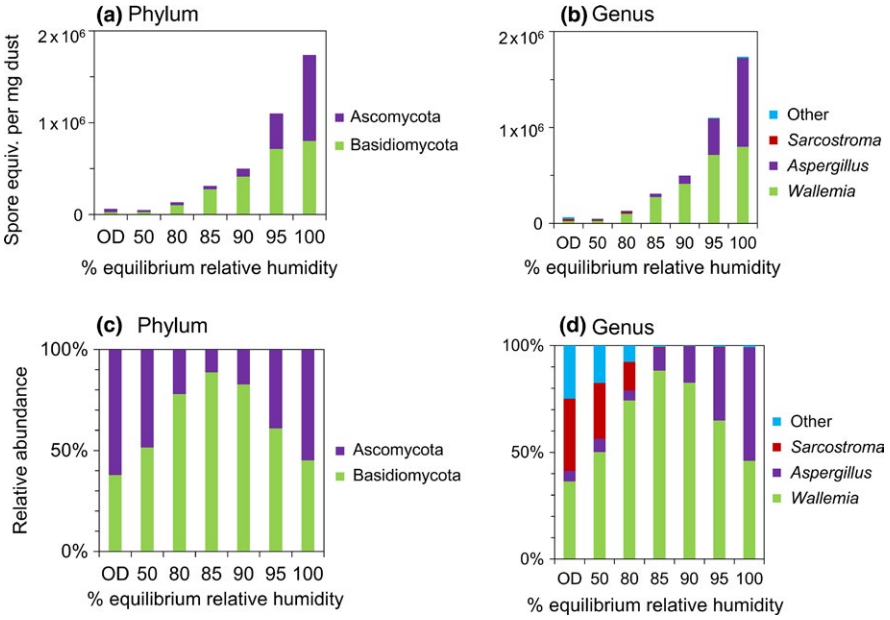

FIGURE 6 Concentrations (a and b) and relative abundances (c and d) of Ascomycota and Basidiomycota at different ERH levels, compared to the original dust (OD). Other phyla not shown constituted a combined 0.01% of detected spore equivalents. ERH, equilibrium relative humidity

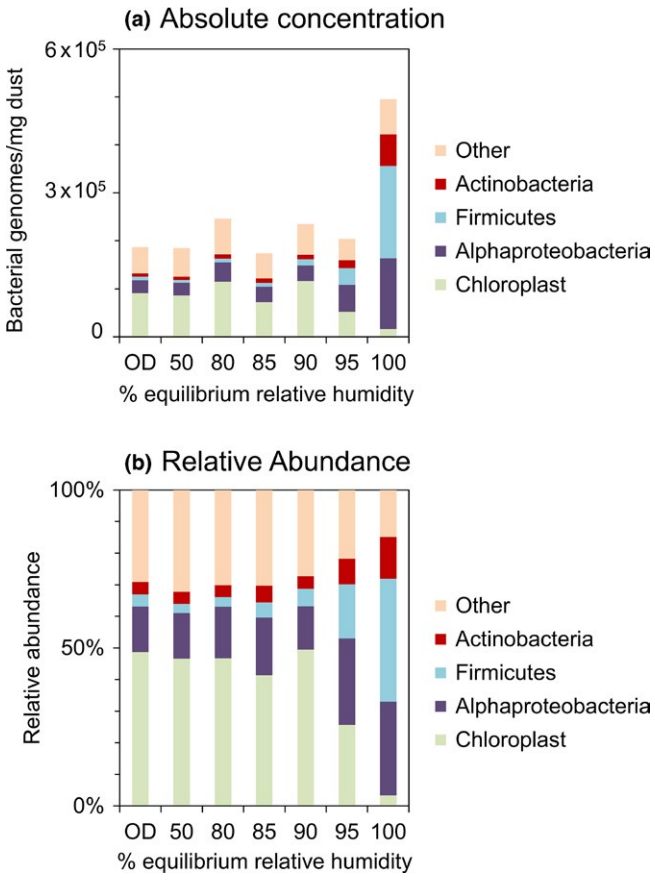

FIGURE 7 Concentrations (a) and relative abundances (b) of major taxonomic groups in bacteria at different ERH levels compared to the original dust (OD). ERH, equilibrium relative humidity

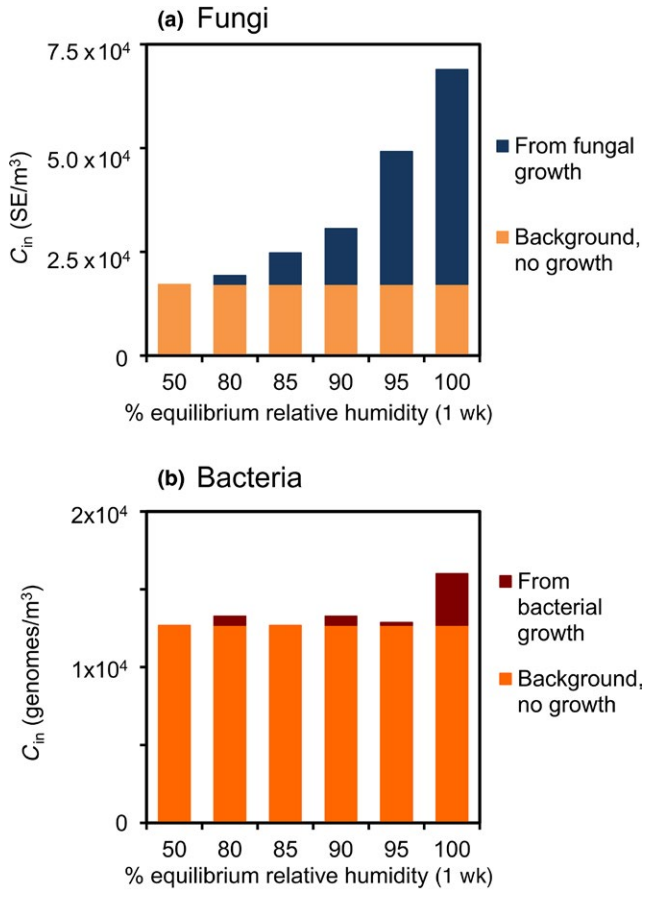

FIGURE 8 Modeled indoor air concentrations of bacteria and fungi. Darker colors denote the contribution of resuspended fungi and bacteria that originated in carpet dust due to 1 wk of growth

community changes at low ERH may be due to processes other than growth, such as decay.

Molecular methods also allow for insights into richness. Previous studies have demonstrated that reported moisture is associated with an increase in fungal richness in floor dust.[25,51–53] This is consistent with the trend observed in the more controlled studies reported here for bacteria, but not for fungi. We note that there are key differences between this and prior building studies. In the prior work, increased

richness with moisture damage was measured in dust samples that were sampled away from the water-damaged building materials. Here, sampling occurred directly on the material (dust) that had increased water activity. One plausible explanation is that the local diversity at the site of moisture damage results in a decrease in diversity due to the large influence of growth of a limited number of taxa, which would result in fewer unique OTUs for the same sequencing depth. In buildings that report water damage, samples from dust that are distant from the damage may show an increased fungal richness due to background dust microbial diversity plus the smaller contribution of water-associated taxa that have been transported to the dust sampling site. In another recent study that sampled in buildings at the site of visible growth, low diversity of fungi on building materials was reported.[48] In our study, the opposite behavior was observed in bacterial richness, possibly due to the lower growth rate and a broader, more even distribution of taxa that grew at 100% RH compared to fungi. Increased bacterial and fungal diversity has been associated with a protective effect in developing asthma,[25,54] and thus, more research into bacterial and fungal diversity in homes with water damage is required to understand the mechanism controlling house dust microbial diversity.

4.2 | Relative humidity in homes

The EPA recommends that relative humidity levels in homes remain below 60%, and ideally between 30 and 50%, to prevent mold growth.[55] While relative humidity in many homes remains below those levels, exceptions can occur.[56] These conditions may tend to occur in homes without air conditioning when the outdoor air is warm and humid. Additionally, if the carpet in a home is cooler than the surrounding air, for example, by coupling directly to a concrete slab, then the relative humidity at the surface of the carpet may be elevated compared to air in the room. Similar conditions may also occur as a result of water damage or intrusion.

4.3 | Implications for human exposure

We used a simple steady-state material balance approach that implemented prior relationships for resuspension factors to demonstrate the potential impacts that growth in carpets may have on aerosol exposure. This understudied exposure is relevant to human health due to the strong association between dampness in buildings and negative respiratory health effects.[57,58]

Under common building occupancy and ventilation scenarios, growth in dust at 85%–100% ERH resulted 25%–95% of the indoor air microbes coming from the floor. Results were lower for bacteria, but still over 10% contribution at 100% RH. Such models are in a nascent stage, and there are several limitations to this approach including uncertainties in resuspension rate coefficients, especially at high RH,[10,59] and a well-mixed assumption. While exact values may not be predictable due to the above-specified limitations in the model, there is strong evidence that human occupancy increases the microbial and total particle load in indoor air through resuspension. From experiments that have measured the bulk resuspension effect, the impact of occupancy and floor dust loading is strong. Resuspension due to walking has been found to explain 24%–55% of variation of 1–25 μm diameter particle number concentration[60] in homes, and occupancy in school classrooms resulting in increased bacterial and fungal concentrations by 66 and 15 times, respectively.[11] Ferro et al.[61] noted that indoor activity including walking and dancing on floors increased PM_{10} concentration by greater than five times and that exposures measured by personal samplers were higher than those by fixed aerosol samplers due to the personal cloud effect.

4.4 | Strengths and limitations

Additional limitations of this study include that this was a laboratory chamber study used to model the indoor environment. While applications to real environments are not direct, the in-depth microbial ecology investigation provides new insight into growth on dust at high RH levels. We were unable to account for diurnal and other typical variations in relative humidity within a home. Results are based on carpet and dust samples from one home, and the microbial communities in other homes differ based on factors such as occupancy, season, and geography. This study reports fungal species identifications to the rank of species. While recent improvements to databases and techniques have improved the accuracy of such classifications, there still may be errors in the precise identification of some of the fungal species.[34] Despite potential errors, these findings are still reported as potentially useful information. Additionally, variation in community composition may affect the measured value of total fungi and bacteria when determined by universal primers with qPCR due to amplification bias, and this may also influence species-specific values because these data were also used to determine that concentration.[39] However, we expect that influence to be small in this study due to the consistent dominance of the communities by a small number of taxa (Figs 6b and 7a). Moisture availability was the limiting factor for growth of microbes on floor dust in this study. Water availability is measured by water activity, which is defined as the ratio of the partial vapor pressure of water in a substance to the partial vapor pressure of pure water and is related to the ERH (ex: 0.95 a_w = 95% ERH). In a substrate or solution, water activity is dependent on salt concentration and composition. In this dust, sodium chloride was the dominant dissolvable salt (Table S2), and at sodium chloride saturation, the water activity is 0.753 at 25°C. Salt composition determines moisture transfer from the air dependent on water activity at saturation,[62] such that dusts from other locations that vary in salt composition may also vary in moisture uptake rates. For instance, dust from southern California influenced by nitrates may differ from dust from the northeastern United States influenced by sulfates.[63,64] Thus, both relative humidity in a building and dust composition may influence microbial growth, and future studies should specifically consider dust with a variety of salt compositions. Finally, the resuspension model could be further refined as noted above, such as by determining the effect of relative humidity level on the resuspension coefficient in this system. The indoor air geometric mean aerodynamic diameters included in the resuspension model were based on studies conducted in schools due

to lack of matching data from homes. Schools may have a different size distribution of resuspended particles than homes.

Strengths of this study include that homogeneous environmental samples were taken from a home and then incubated under controlled conditions at constant temperature. The use of molecular techniques allows for unprecedented quantification and identification of the microbial communities in floor dust at elevated ERH levels. Reported values represent concentration (instead of relative abundance), and we considered both bacterial and fungal communities. The resuspension study brings an important indoor air and exposure context to the fungal and bacterial growth results.

5 | CONCLUSIONS

The information in this study was derived from dust and carpet from a single home in the northeastern United States, and we must be cautious generalizing. While we expect to find similar behavior in other homes, different homes contain different microbial communities. Many factors influence an indoor microbial community, including home characteristics, presence of pets, the occupants of the home, and geography.[1–4,65] Upon exposure to elevated relative humidity, there may be variation in resuspension from different carpets, different initial communities, different final communities, and different dominant species.

The new information provided here on the growth and emissions of microbes in dust contributes to our understanding of how moisture shapes the indoor microbiome. Microbial growth occurred in carpet dust from this home at ≥80% relative humidity for fungi and 100% for bacteria. This growth results in a significant increase in concentration and changes the type and diversity of dust microbes. At sustained elevated RH, growth in floor dust may contribute more than half of a room's airborne microbial concentration and thus represents an understudied potential source of human exposure. These results emphasize the role of microbial growth in the assembly of a building's microbiome.

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